IMMUNOLOGICAL SCIENCES Product Data Sheet

CO-K102-M Hydrogen Peroxide (H2O2) Colorimetric Assay Kit

Method: Colorimetric method

Specification：96T (80 samples)

Measuring instrument：Microplate reader（402-407 nm）

Detection range：0.41-125 mmol/L

Intended use

This kit can be used to measure the H_2O_2 content in serum, plasma, urine, tissue and cells samples.

Detection principle

Hydrogen peroxide (H_2O_2) is a metabolic by-product of reactive oxygen species, which is not only a signal molecule in cells, but also a source of oxidative stress. H_2O_2 is an important regulatory factor of eukaryotic signal transduction involved in cell proliferation, differentiation and migration. However, abnormal H_2O_2 can lead to oxidative cell damage and disease, such as cancer, atherosclerosis, osteoporosis and neurodegenerative diseases.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	6 mL ×1 vial	12 mL ×1 vial	2-8 ℃, 12 months
Reagent 2	Ammonium Molybdate Reagent	6 mL ×1 vial	12 mL ×1 vial	2-8 ℃, 12 months
Reagent 3	1 mol/L H_2O_2 Standard	1 mL ×1 vial	1 mL ×2 vials	2-8 ℃, 12 months
	Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (402-407 nm, optimum wavelength: 405 nm), Micropipettor, Centrifuge, Incubator, Vortex mixer

Reagents:

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 1 mol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0, 10, 20, 40, 60, 80, 100, 125 mmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (mmol/L)	**0**	**10**	**20**	**40**	**60**	**80**	**100**	**125**
1 mol/L standard (μL)	0	10	20	40	60	80	100	125
Double distilled water (μL)	1000	990	980	960	940	920	900	875

Sample preparation

① Sample preparation

Serum, plasma and urine: detect directly. If not detected on the same day, the serum or plasma can be stored at -80 ℃ for a month.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 20 mg tissue in 180 μL PBS (0.01 M, pH 7.4) with a dounce homogenizer at 4 ℃.

④ Centrifuge at 10000 ×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant

Cell (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1 ×10^6 cells).

② Wash cells with PBS (0.01 M, pH 7.4).

③ Homogenize 1 ×10^6 cells in 300-500 μL PBS (0.01 M, pH 7.4) with a ultrasonic cell disruptor at 4℃ .

④ Centrifuge at 10000 ×g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant

② Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Rat serum	1
Mouse serum	1
Mouse plasma	1
Porcine serum	1
Human urine	1
Cell homogenate	1
10% Plant tissue homogenate	1
10% Rat heart tissue homogenate	1
10% Rat brain tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat liver tissue homogenate	1

Note: The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

If the concentration of H_2O_2 in the sample is too high, please dilute the samples appropriately. If the concentration is too low, the sampling volume of the sample should be increased, and the sampling volume of standard and double distilled water should be increased equally at the same time.

Operating steps

① Add 100 μL of buffer solution to standard well and sample well, preheat at 37 ℃ for 10 min.

② Standard well: add 15 μL of standards with different concentrations to the corresponding wells.

Sample well: add 15 μL of sample to the corresponding wells.

③ Add 100 μL of ammonium molybdate reagent and mix fully.

④ Mix for 5 s with microplate reader and stand for 10 min at room temperature.

⑤ Measure the OD values of each well at 405 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample and other liquid samples:

$$H_2O_2 \text{ content (mmol/L)} = (\Delta A_{405} - b) \div a \times f$$

2. Tissue and cell samples:

$$H_2O_2 \text{ content (mmol/gprot)} = (\Delta A_{405} - b) \div a \div C_{pr} \times f$$

[Note]

ΔA_{405}: Absolute OD ($OD_{Sample} - OD_{Blank}$).

f: Dilution factor of sample before test.

C_{pr}: Concentration of protein in sample, gprot/L.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	5.50	48.60	102.30
%CV	3.5	3.1	3.0

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (mmol/L)	5.50	48.60	102.30
%CV	3.2	3.6	4.0

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 105%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (mmol/L)	16.5	55.8	95
Observed Conc. (mmol/L)	17.7	58.0	98.8
Recovery rate (%)	107	104	104

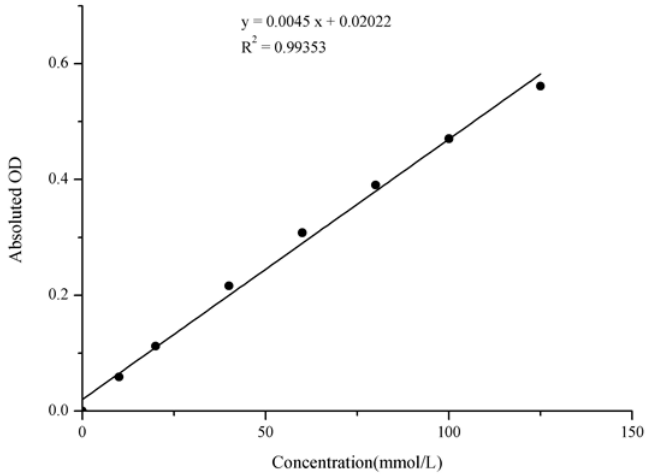
Sensitivity

The analytical sensitivity of the assay is 0.41 mmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (mmol/L)	0	10	20	40	60	80	100	125
OD value	0.072	0.132	0.188	0.287	0.380	0.468	0.543	0.636
	0.076	0.134	0.184	0.293	0.384	0.460	0.545	0.634
Average OD	0.074	0.133	0.186	0.290	0.382	0.464	0.544	0.635
Absoluted OD	0	0.059	0.112	0.216	0.308	0.390	0.470	0.561



Appendix II Example Analysis

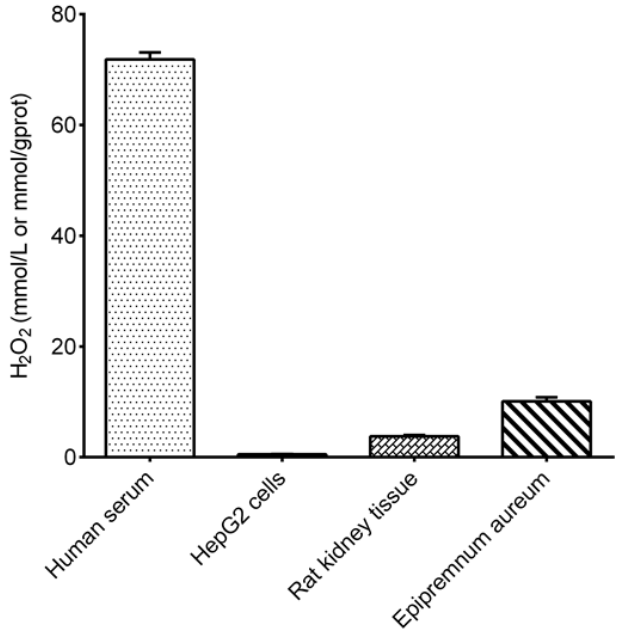
Example analysis:

Take 15 μL of human serum sample and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.0047 x + 0.0223, the average OD value of the sample is 0.435, the average OD value of the blank is 0.075, and the calculation result is:

$$H_2O_2 \text{ content (mmol/L)} = (0.435 - 0.075 - 0.0223) \div 0.0047 \times 1 = 71.85 \text{ (mmol/L)}$$

Detect human serum, HepG2 cell homogenate (the concentration of protein in sample is 5.00 gprot/L), 10% rat kidney tissue homogenate (the concentration of protein in sample is 6.57 gprot/L), 10% epipremnum aureum tissue homogenate (the concentration of protein in sample is 0.99 gprot/L) according to the protocol, the result is as follows:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Immunological Sciences will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.